Cellular Advantages to Signaling in a Digital World

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A newly revealed cellular strategy for modularizing function inspires engineers.

Complex machines have evolved alongside the human race thanks to centuries of tinkering by engineers. To make machines more controllable and robust to diverse settings, engineers have discovered modular designs (connecting smaller modules into larger systems) and identified many useful elements and design principles. One such principle, digital behavior, ensures an all-or-none response to an input, yielding solutions that are robust to varying input signal strength and contextual perturbations. A great example is timekeeping. Ancient analog solutions, such as sundials and hourglasses that are sensitive to perturbations (e.g., from a rocking table), gave way to mechanical clocks with discrete elements like pendulum oscillators, and eventually to quartz piezoelectric devices with modules that are connected entirely digitally (Figure 1).

Cells, the complex machines produced by Nature's tinkering, are composed of intricate networks of interacting molecules that enable them to monitor their environment and make sophisticated decisions. Understanding how these cellular networks function and illuminating their design principles has been a goal of top-down approaches like systems biology and bottom-up approaches like synthetic biology. Both have invoked the appealing notion that cells are organized by functional modules, that is, discrete sub-networks that carry out separable functions; this would then allow higher-order functions to emerge by simply connecting together modules, much like in engineering (Hartwell et al., 1999). Yet, systems biology has faced difficulty disentangling networks into constituent modules (Mitra et al., 2013), while in synthetic biology, connecting synthetic modules into larger functional networks has proved quite challenging (Cardinale and Arkin, 2012; Del Vecchio et al., 2016; Khalil and Collins, 2010). Therefore, the extent to which biological networks can function in a modular sense remains an open question. Moreover, if there is modularity, how is separation achieved?

Atay et al. (2016) pose these questions in the context of a classic cell-fate decision: the decision for budding yeast to arrest cell cycle during mating. This begins with pheromone encounter, inducing a signaling cascade from which follows cell-cycle arrest, morphological changes, and ultimately mating with a partner cell. The decision to arrest is not made lightly, and requires highly accurate processing and memory of pheromone signals in the context of cell-cycle differences.

At the molecular level, the upstream mating response (or pheromone) pathway interfaces with the downstream cell-cycle regulatory pathway through a feedforward loop (FFL) (Figure 1). To arrest cell cycle, the mating response pathway triggers the FFL to activate a cell-cycle inhibitor, Far1, via two mechanisms: Far1 phosphorylation (fast) and Far1 synthesis (slow). To re-enter the cell cycle, cyclin-Cdk complexes inhibit the mating cascade by triggering degradation of Far1 and inhibiting its synthesis. This double negative feedback between Far1 and cyclins ensures that cells arrest in G1 phase of the cell cycle.

The authors hypothesized that, when boiled down, this sophisticated connectivity between the two pathways may uncover a means for insulating the FFL motif from cell-cycle effects and other perturbations that would otherwise jeopardize this critical decision. Specifically, extensive prior work (Doncic et al., 2011, 2015; Doncic and Skotheim, 2013) pointed to positive feedbacks on cyclins regulating cell-cycle re-entry. These positive feedbacks effectively "digitize" the re-entry switch, converting an analog input (cyclin-Cdk activity) into an ON/OFF digital output (re-entry). A digital re-entry switch means that, from the standpoint of the FFL, the activity of the cell-cycle pathway is negligible (OFF) most of the time (outside of commitment to division), effectively allowing the FFL to operate as an isolated module. This switch-like interpretation of cell-cycle pathway activity can thus promote separation of timescales between *sustained* arrest and *rapid* re-entry.

Other mechanisms have been proposed for insulating molecular modules, including: spatial separation of components within complexes or to specific parts of the cell (Doncic et al., 2015; Bhattacharyya et al., 2006), and timescale separation at which molecular events occur (e.g., circuits that utilize both fast phosphorylation and slow transcription, Mishra et al., 2014). These findings altogether are suggestive of general design principles for insulating modules in biology akin to those guiding engineers in other disciplines. In order to expand the scope of these findings and reinforce the generality of the principle, two avenues of study are immediately apparent.

In one avenue, systems biology can use this principle to guide efforts in search of

modular structure within natural biological networks. Positive feedback and other motifs predicted to drive switch-like responses are pervasive in regulatory networks (Alon, 2007), which supports the compelling idea that many more examples of module insulation via non-linear connections await discovery. Indeed, it will be intriguing to see to what extent this serves as a broader organizational principle of cellular networks.

Identifying modular structure in biological networks also provides insight into their evolution. Modularity has been suggested to enable faster evolution of networks, as new functions can emerge by recombining existing components with new connections (Bhattacharyya et al., 2006). Insulation may play a key role in this process, ensuring that evolving subnetworks remain modular and make robust all-or-none decisions.

In the other avenue, synthetic biology can harness the principle of insulation via digital connections directly, by building non-linear connections between discrete modules in a network. If successful, this could help synthetic biologists tackle the challenge of predictably constructing larger, more complex biological systems guided only by models of constituent modules. In one of the more state-of-the-art and rigorous tests of this idea, researchers recently developed technology for fully automated design of genetic circuits at the module level (Nielsen et al., 2016). The platform draws on a database of genetic logic gates implemented with bacterial repressors (the modules) to design larger genetic circuits (that implement steady-state ON/OFF outputs). When a large number of designs outputted by the software were constructed in *E. coli*, many of the circuits functioned reliably, though gate modules needed to be effectively insulated and reliability dropped as systems grew in size.

Advancing these efforts will likely require engineering new types of molecular connectivity. This means going beyond the paradigm of rigid "parts," like simple bacterial repressors, and instead engineering new "parts" that can flexibly implement non-linearity, different regulatory timescales (e.g., programmable phosphorylation), and complex molecular assembly. As evidenced in Atay et al. and other works, these mechanisms are important in connecting natural biological modules, and synthetic designs that can tune these parameters might prove more successful at the network scale.

Insulation via digital connections might also permit synthetic biologists to effectively integrate into endogenous cellular networks, potentially obviating the need to model the larger cellular context into which the synthetic systems are embedded, leveraging natural pathways, and easing the burden of finding and characterizing extensive libraries of orthogonal parts. Ideally, one would be able to share components between modules and repurpose modules or elements from the host network. This would ease the metabolic load incurred by exogenous protein expression especially in the case of larger networks with many components. However, naively using a synthetic component to connect to and regulate many downstream pathways in the cell can lead to loading effects that negatively impact the upstream module function, an effect termed retroactivity.

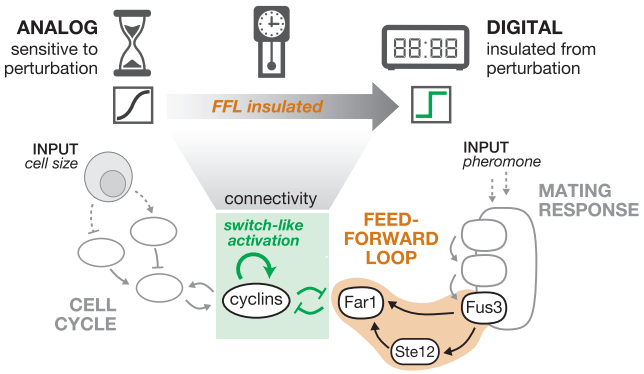

Figure 1. Digital Connectivity Has Enabled the Development of Modules and Machines with Robust Function in the Face of Contextual Perturbations

Atay et al. provide evidence that non-linear molecular connections can also discretize modules in biological networks. In the yeast mating decision, a feedforward loop (FFL, orange) controlling cell-cycle arrest is fully insulated from cell-cycle perturbations and dynamics. Insulation is achieved with switch-like connectivity to the cell cycle using positive feedback switches on cyclins (green box).

Creative synthetic strategies have begun to emerge whereby timescale separation modules, such as engineered phosphotransfer modules, are inserted into transcriptional circuits to mitigate these effects (Mishra et al., 2014).

Pervasive use of digital connectivity to isolate and insulate modules within biological networks has important implications for both modeling and design in systems and synthetic biology, reflecting the centrality of this design principle in other disciplines. The evidence provided by Skotheim and colleagues bolsters this emerging theory and makes an important contribution to our understanding of the cellular machine.

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